CLAIMS

What is claimed is:

1. A method, implemented on a computer, of predicting the relative reaction velocities of (i) a first reaction pathway in which a substrate reacts in the presence of a cytochrome P450 enzyme to form a product and (ii) a second pathway in which hydrogen peroxide is produced from a complex of the cytochrome P450 enzyme and the substrate in a manner that regenerates the substrate without producing the product, the method comprising:

(a) analyzing the molecular structure of the substrate to identify a structural feature that affects the rate at which hydrogen peroxide is formed in the active site of the cytochrome P450 enzyme; and

(b) predicting the relative contributions of the first and second reaction pathways based on the identity of the structural feature, to thereby predict the relative reaction velocities.

2. The method of claim 1, wherein the structural feature at least partially excludes at least one of water and hydrophilic components from a defined region of the cytochrome P450 enzyme's reactive site.

3. The method of claim 1, further comprising predicting the relative contribution of a water decoupling pathway for each reactive site.

4. A computer program product comprising a machine readable medium on which is provided instructions for predicting the relative reaction velocities of (i) a first reaction pathway in which a substrate reacts in the presence of a cytochrome P450 enzyme to form a product and (ii) a second pathway in which hydrogen peroxide is produced from a complex of the cytochrome P450 enzyme and the substrate in a manner that regenerates the substrate without producing the product, the instructions specifying a method comprising:

(a) analyzing the molecular structure of the substrate to identify a structural feature that affects the rate at which hydrogen peroxide is formed in the active site of the cytochrome P450 enzyme; and

(b) predicting the relative contributions of the first and second reaction pathways based on the identity of the structural feature, to thereby predict the relative reaction velocities.

5. The computer program product of claim 4, wherein the structural feature at least partially excludes at least one of water and hydrophilic components from a defined region of the cytochrome P450 enzyme's reactive site.

6. The computer program product of claim 4, wherein the method further comprises predicting the relative contribution of a water decoupling pathway for each reactive site.

7. A method of developing a model for predicting the rate at which a substrate is metabolized by a cytochrome P450 enzyme, the method comprising:

(a) using stoichiometric data to characterize

(i) the reaction rate of a first reaction pathway in which the substrate reacts in the presence of the cytochrome P450 enzyme to form a product

(ii) the reaction rate of a second pathway in which hydrogen peroxide is produced from a complex of the cytochrome P450 enzyme and the substrate in a manner that regenerates the substrate without producing the product, and

(iii) the reaction rate of a third pathway in which water is produced from a second complex of the cytochrome P450 enzyme and the substrate in a manner that regenerates the substrate without producing the product; and

(b) specifying one or more reaction rate expressions or rate constants derived using the stoichiometric data for the first, second, and third pathways, to thereby allow a test substrate to be evaluated by said reaction rate expressions or rate constants.

8. The method of claim 7, wherein using stoichiometric data to characterize the reaction pathways comprises using stoichiometric data for multiple substrates.

9. The method of claim 7, wherein the stoichiometric data is obtained from experiments that measure the concentration of NADPH.

10. The method of claim 9, wherein the experiments also measure the concentration of hydrogen peroxide.

11. The method of claim 9, wherein the experiments also measure the concentration of at least one of oxygen and a product of the metabolism.

12. For a catalytic cycle involving an enzyme and a substrate, which catalytic cycle has (a) a first reaction pathway in which a substrate reacts in the presence of the enzyme to form a product and (b) a second pathway in which water is produced from a complex of the enzyme and the substrate in a manner that regenerates the substrate without

producing the product, a method of determining the relative contribution of the second pathway, the method comprising:

determining a change in concentration of NADPH during a reaction involving at least the following reactants: NADPH, the enzyme, and the substrate;

determining a change in concentration of oxygen during the reaction; and

estimating the relative contribution of the second pathway based upon the relative values of the change in concentration of NADPH and the change in concentration of oxygen.

13. The method of claim 12, wherein the change in oxygen concentration is determined without directly measuring oxygen concentration during the reaction.

14. The method of claim 12, wherein estimating the relative contribution of the second pathway comprises determining the excess amount of the change in concentration of NADPH over the change in concentration of oxygen.

15. For a catalytic cycle involving an enzyme and a substrate, which catalytic cycle has (a) a first reaction pathway in which a substrate reacts in the presence of the enzyme to form a product and (b) a second pathway in which hydrogen peroxide is produced from a complex of the enzyme and the substrate in a manner that regenerates the substrate without producing the product, a method of determining the relative contribution of the second pathway, the method comprising:

conducting a first reaction involving at least the following reactants: NADPH, the enzyme, and the substrate;

determining a change in concentration of oxygen during the first reaction;

conducting a second reaction involving at least the following reactants: NADPH, the enzyme, the substrate, and catalase;

determining a change in concentration of oxygen during the second reaction;

comparing the change in oxygen concentrations in the first and second reactions to determine the amount of hydrogen peroxide produced via the catalytic cycle.

16. The method of claim 15, wherein the first and second reactions are conducted simultaneously.

17. The method of claim 15, wherein the change in oxygen concentration is determined by measuring the change in NADPH concentration.

18. The method of claim 15, wherein comparing the change in oxygen concentrations in the first and second reactions comprises determining the excess amount of the change in concentration of oxygen in the second reaction over the change in concentration of oxygen in the first reaction.

19. A method generating a model of substrate metabolism using the rate or amount of water formation for a substrate molecule in a cytochrome P450 catalyzed reaction, the method comprising:

(a) measuring the rate or amount of consumption of oxygen;

(b) measuring the rate or amount of consumption of NADPH;

(c) comparing the rates or amounts of consumption of oxygen and NADPH to determine the rate or amount of water formation from a water decoupling branch pathway for the substrate molecule in the cytochrome P450 catalyzed reaction, and

(d) generating a general model of substrate metabolism based on molecular structure.

20. The method of claim 19, further comprising calculating a reaction rate for the water-decoupling pathway of the substrate molecule.

21. The method of claim 19, wherein the concentration of oxygen is reduced to a reaction-limiting amount prior to the cytochrome P450 catalyzed reaction.

22. The method of claim 19 wherein (a) (b) and (c) are repeated for at least two substrate molecules.

23. The method of claim 19 wherein (a) (b) (and (c) are repeated for a class of substrate molecules.

24. The method of claim 19 wherein the rate or amount of oxygen consumption is measured using a ruthenium complex matrix.

25. The method of claim 20 wherein the reaction rate is calculated based on the difference between oxygen and NADPH consumption.

26. A method for calculating the rate or amount of consumption of peroxide formation for a substrate molecule in a cytochrome P450 catalyzed reaction, the method comprising:

(a) measuring the rate or amount of consumption of oxygen;

(b) measuring the rate or amount of consumption of NADPH;

(c) adding catalase to the reaction; and

(d) measuring the rate or amount of formation of oxygen.

27. The method of claim 26 further comprising calculating a reaction rate for the peroxide-decoupling pathway of the substrate molecule.

28. The method of claim 26 wherein the amount of oxygen is reduced to a reaction-limiting amount.

29. The method of claim 26 wherein (a) (b) (c) and (d) are repeated for at least two substrate molecules.

30. The method of claim 26 wherein (a) (b) (c) and (d) are repeated for a class of substrate molecules.

31. The method of claim 26 further comprising generating a general model of substrate metabolism based on molecular structure.

32. The method of claim 26 wherein the rate or amount of oxygen consumption is measured using a ruthenium complex matrix.

33. A method for modeling reaction rates of cytochrome P450 metabolism for substrate molecules, the method comprising:

(a) receiving or generating a peroxide-decoupling rate and a molecular structure for a substrate molecule; and

(b) receiving or generating a water-decoupling rate and a molecular structure for a substrate molecule.

34. The method of claim 33 wherein (a) and (b) are repeated for at least two substrate molecules.

35. The method of claim 33 wherein (a) and (b) are repeated for a class of substrate molecules.

36. The method of claim 33 further comprising receiving a product-formation rate and a molecular structure for a substrate molecule.

37. A method for modeling absolute rates of cytochrome P450 metabolism for substrate molecules, the method comprising:

creating a computational model for peroxide-decoupling rate based on substrate molecule structures; and

creating a computational model for water-decoupling rate based on substrate molecule structures.

38. The method of claim 37 further comprising creating a computational model for product-formation pathway rate based on substrate molecule structures.

39. A method of predicting the relative reaction velocities of (a) a first reaction pathway in which a substrate reacts in the presence of a cytochrome P450 and (b) a second pathway the method comprising:

analyzing the molecular structure of the substrate to determine if it possesses a particular structural feature; and

predicting whether the first reaction pathway is preferred when the substrate possesses said structural feature.

40. The method of claim 39 wherein the first reaction pathway is a product-formation pathway.

41. The method of claim 39 wherein the first reaction pathway is a peroxide-decoupling pathway.

42. The method of claim 39 wherein the particular structural feature is one that excludes water from the substrate reactive site.

43. The method of claim 39 wherein the particular structural feature is one that does not exclude water from the substrate reactive site.

44. The method of claim 39 wherein the particular structural feature is hydrophobic.

45. The method of claim 39 wherein the particular structural feature is hydrophilic.

46. A computer program product comprising a machine readable medium on which is provided instructions for executing a method of predicting the relative reaction velocities of (a) a first reaction pathway in which a substrate reacts in the presence of a cytochrome P450 and (b) a second pathway the method comprising:

analyzing the molecular structure of the substrate to determine if it possesses a particular structural feature; and

predicting whether the first reaction pathway is preferred when the substrate possesses said structural feature.

47. The computer program product of claim 46 wherein the first reaction pathway is a product-formation pathway.

48. The computer program product of claim 46 wherein the first reaction pathway is a peroxide-decoupling pathway.